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Toxicity of Sewage Sludge to 'Rhepoxynius abronius', a Marine Benthic Amphipod

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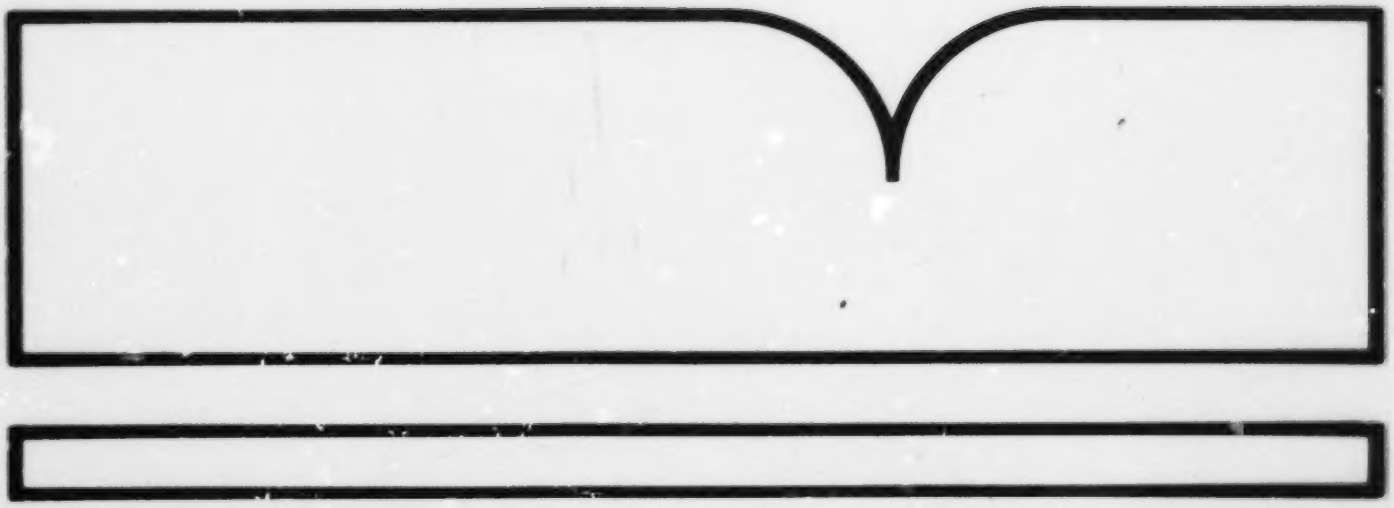

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16. ABSTRACT

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Toxicity of Sewage Sludge to *Rhepoxynius abronius*, a Marine Benthic Amphipod

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Abstract. Relative toxicity of sewage sludges from six treatment plants was determined by the LC50 of sludge-sediment mixtures to the infaunal marine amphipod, *Rhepoxynius abronius*. LC50s were measured as the increase in the percent total volatile solids (TVS) of the mixture due to the addition of sludge required to kill 50% of the amphipods during a 10-day exposure. LC50s ranged from 2.83% TVS addition for sludge from the small, domestic community of Waldport, Oregon to <0.1% TVS addition for metropolitan, more industrialized sources in Los Angeles, California. The toxicity of the sludge-sediment mixtures is attributed primarily to chemical contamination rather than organic enrichment. Rank correlations between toxicity and specific chemicals were usually not statistically significant, indicating that different combinations of stresses involving multiple or unmeasured factors were probably responsible for observed effects. Integrative measures of contamination (oil/grease concentration; consensus ranking of contamination based on 15 parameters) were significantly correlated with *R. abronius* survival indicating that the more toxic sludges had a higher overall level of contamination.

The toxicity of marine pollutants to benthic organisms has recently been identified as a priority research topic necessary to establish criteria for the disposal of waste materials in coastal waters (Goldberg 1979). Sewage effluents and sludges discharged from barges and ocean outfalls have contaminated the seabed and altered benthic communities in many coastal areas (Pearce 1972; Anger 1975; Pearson and Rosenberg 1978; Young *et al.* 1978; Botton 1979; Bascom 1982; Steimle *et al.* 1982). To improve methods of predicting the ecological effects of sewage disposal, a bioassay method was developed for the toxicity of sewage particulates to a benthic infaunal amphipod, *Rhepoxynius abronius* Barnard.

The bioassay design employed in the research is to organically enrich unpolluted sediment by adding sewage sludge in a series of increasing concentrations. Toxicity is defined by the 10-day LC50 for total volatile solids added to the sediment, *i.e.*, the enrichment level that causes the acute mortality of one-half the test organisms during a 10 day exposure. To determine the efficacy of this method in comparing the toxicity of sewage from different sources and treatments, bioassays were conducted of sewage sludge from six plants serving small, principally domestic to large, more industrialized communities. To discriminate between chemical contamination and organic enrichment as causes of toxicity, relations were examined between LC50 values and organic and pollutant concentrations in the sludge-sediment mixtures.

Materials and Methods

The sludge bioassay is an adaptation of a method used by regulatory agencies to determine the acceptability of dredged material for ocean disposal (EPA/COE 1977). The test species, *Rhepoxynius abronius* is a burrowing, infaunal amphipod and its dependency on interstitial water and sediment quality appears to contribute to its sensitivity in benthic bioassays. Swartz *et al.* (1979), who identified this species as *Paraphoxus epistomus*, found it to be more sensitive than other benthic invertebrates to contaminated sediment. The scale of the present design is much smaller than that of the dredge material procedure (1 L vs 25 L bioassay container) and the seawater system is static rather than flow-through. These factors reduce logistic difficulties and allow for greater replication and precision (Swartz *et al.*, in preparation).

Table 1. Sources of sewage sludge

Source	Experiment number	Treatment of sample used in bioassay	Remarks
Waldport, Oregon	1	Secondary treatment (activated sludge), digested sludge	Residential population 2,500, light commercial, tourism
Newport, Oregon	1	Secondary treatment (trickling filter), digested sludge	Residential 10,000, commercial, seafood processing, tourism
City of Los Angeles, California (Hyperion)	2	Primary treatment, digested sludge	Metropolitan, commercial, industrial
Los Angeles County, California (White's Point)	2	Advanced primary treatment (polymer addition, fine-mesh screening), digested sludge	Metropolitan, commercial, highly industrial
Middlesex County, New Jersey (Sayreville)	3	Secondary treatment, digested sludge	Metropolitan, highly industrial
New York City, Staten Island (Oakwood Beach)	3	Secondary treatment (step aeration activated sludge), digested sludge	Relatively new system, mostly residential

Collection of Sediment and Amphipods

Rhepoxynius abronius and clean test sediment (control) were collected with a small dredge from lower Yaquina Bay, Oregon (44° 37′ 33″ N, 124° 02′ 58″ W). Adult amphipods (~4 mm total length) were removed by gently sieving sediment through a 1.0 mm screen. Amphipods were picked from the material retained on the screen and maintained in sieved sediment until the bioassay was initiated 3 or 4 days later. A portion of the sediment that passed through the screen was allowed to settle and then stored at 4°C for a maximum of three days before it was used to make the sludge-sediment mixtures. The sediment was a well-sorted, fine-grained sand with an equivalent spherical diameter of 0.165 mm.

Preparation of Sludge-Sediment Mixtures

Sewage sludges used to organically enrich the Yaquina Bay sediment were collected from six primary or secondary treatment plants in Oregon, California, New Jersey, and New York (Table 1). None of the sludges was chlorinated. All were collected within four days of the initiation of the bioassay and stored at 4°C.

Sludge was concentrated by centrifugation in 250 ml centrifuge bottles at 5,000 rpm (4080 × G) for 10 min. This resulted in a black, mayonnaise-like sludge "cake" that was easily mixed by spatula with the control sediment.

The level of organic enrichment was expressed as the increase in percent total volatile solids of the control sediment due to the addition of the sludge. The percent total solids (TS) and total volatile solids (TVS) were determined for the control sediment and sludge cake. TS was calculated as the percent of the initial wet weight of a sample remaining after drying at 105°C for 12 hr. TVS was calculated as the percent weight loss of the dried sample after it was heated to 550°C for 1 hr. These data were used to calculate the proportions of wet sediment and sludge cake required to prepare a geometric series of TVS added to the control sediment.

The first two experiments (Oregon and California sludges, Table 1) were done with seven bioassay treatments: control sediment with no sludge addition and six sludge-sediment mixtures whose TVS was equal to the background TVS of the control sediment plus 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0%. In the third experiment (New York and New Jersey sludges) a 0.0625% TVS addition was included in the geometric series as an eighth treatment. The background TVS of the control sediments were 1.76, 1.28, and 1.37% for experiments 1, 2, and 3, respectively.

Bioassay Procedure

Bioassay chambers (1 L glass beakers) were filled to a 2-cm depth with test sediment. A plastic disc was placed on the sediment surface to minimize disturbance as 600 ml of seawater (5 μ filtered, 25‰ salinity) were added to the chamber. Each beaker was covered with Parafilm and gently aerated through a 1 ml pipette that extended no closer than 2 cm from the sediment surface. The chambers were placed in a 15°C water bath and allowed to equilibrate overnight.

Five replicate bioassay chambers were prepared for the control and each sludge-sediment mixture with ≤1.0% TVS enrichment. The bioassay samples for the 2% and 4% TVS enrichment were not replicated because of the difficulty of extracting a sufficient quantity of sludge solids. The bioassay was initiated by randomly distributing 20 *R. abronius* to each bioassay chamber along with 200 ml of seawater (final volume 800 ml seawater overlying sediment). Typically, the amphipods quickly swam to the bottom and burrowed into the sediment. After 10 days, the contents of the beakers were sieved through a 0.5 mm screen and survivors counted and placed in a fingerbowl with control sediment and fresh seawater. The number that failed to rebury in the sediment after one hr was recorded.

Chemical analyses showed that the mean measured TVS was 108.9 ± 9.5% ($\bar{x} \pm S_x t_{0.5}$) of the nominal concentration. The confidence limits on this mean were widest for lower enrichment levels that were close to the precision of the TVS analysis. Measured TVS was 99.8 ± 3.5% of the nominal concentration for

enrichment levels ≥0.5% TVS. Nominal concentrations were therefore used to calculate LC50 values.

LC50 values were calculated by probit analysis involving linear regression of percent mortality against the logarithm of the percent total volatile solids addition (Finney 1971).

Analytical Chemistry

Chemical parameters were measured in 1-L bioassay beakers containing the sludge-sediment mixtures, but no amphipods. Two beakers were setup at each sludge concentration for chemical analyses. Except for the metals, data were collected for all chemical parameters at each sludge concentration at the initiation (day 0) and termination (day 10) of the bioassays. At least six sediment samples from each TVS enrichment level were analyzed for metals. Metal analyses were conducted for all sediment samples from the 2% TVS treatments (12 samples: 6 sludge sources × 2 sampling times).

Dissolved oxygen (DO) and pH were measured daily in the water column with a YSI Model 57 DO meter and Corning pH electrode/Orion Ionalyzer, respectively. Eh was measured daily by inserting a Pt/calomel electrode 1 cm beneath the sediment surface. Interstitial water was collected by carefully decanting the water overlying the sediment surface and then allowing interstitial water to drain into a depression created in the sediment. The interstitial water sample was withdrawn into a syringe and passed through a 0.45 μm Millipore filter into: 1) autoanalyzer sample cups containing $HgCl_2$ (40 mg/L) as a preservative for NH_3-N analysis, and 2) glass vials containing sulfide antioxidant buffer for soluble sulfide analysis. Ammonia nitrogen was analyzed by the automated indophenol blue method (EPA 1979). Free sulfides were measured by cadmium nitrate titration using a sulfide electrode.

Samples were taken for sediment analysis after mixing sediment in experimental beakers with a Teflon spoon. Samples for metal analyses were placed in capped plastic containers and stored at 4°C. Metals were extracted by shaking with 0.01 N HCl for 2 hr, and analyzed by flameless atomic absorption spectrophotometry for Cd, Pb, Zn, Cr, Cu, Ni, and Ag. The 0.01N HCl extraction solution is probably representative of the lowest pH values of digestive fluids and this leaching procedure can be used to estimate the portion of the total metal content of a waste that is biologically available (Atlas *et al.* 1980). The mean percent of the metal content of the 2% TVS enriched sediment removed by the 0.01N HCl extraction relative to that removed by concentrated HNO_3 varied among metals: Cd (77%), Pb (6), Zn (28), Cr (4), Cu (2), Ni (19), and Ag (20). Samples for organic carbon and oil/grease analyses were freeze-dried in glass containers. Total organic carbon was measured by an Oceanographic International Corp. carbon analyzer. Total oil/grease was extracted with freon and analyzed by infrared spectrophotometry (APHA 1980). The hydrocarbon oil/grease fraction was separated from the freon extract by silica gel and reanalyzed by infrared spectrophotometry (APHA 1980).

Replicate chemical analyses were conducted to estimate the precision of the analytical methods. The mean coefficient of variation for 17 duplicate or triplicate metal analyses was 13.2%. The mean coefficient of variation for 16 duplicate oil/grease analyses was 16.7%. These precision estimates are much smaller than the range of metal and oil/grease concentrations in the sludge-sediment mixtures.

Kendall's coefficient of rank correlation (Sokal and Rohlf 1969) was used to determine correlations between toxicity (LC50) and concentrations of chemical contaminants in the sewage sludge-sediment mixtures. The sludge-sediment mixtures were first ranked in order of decreasing toxicity as determined by the LC50. They were then ranked in order of decreasing pollutant concentrations. This second ranking was done separately for 15 parameters (Eh, percent solids, total volatile solids, total organic carbon, ammonia nitrogen, free sulfides, hydrocarbon oil/grease, total oil/grease, Cd, Pb, Zn, Cr, Cu, Ni, and Ag). Ranks for Eh and percent solids were reversed because values for these parameters are inversely proportional to organic enrichment and contamination. Except for the 2% TVS enrichment level, rank correlations for metals are based on fewer samples (6–9) per enrichment level than other chemical parameters (12 samples). Concentrations of these parameters in each sludge-sediment mixture at both the initiation and termination of the bioassay were included in the ranking. The 6 total volatile solids enrichment levels common to all bioassays (0.125, 0.25, 0.5, 1.0, 2.0, 4.0% TVS enrichment) provided independent tests of the toxicity-contamination correlation. Thus, 90 correlation coefficients (15 parameters × 6 enrichment levels) were calculated. In addition, for the 2% enrichment level we tested the correlation between toxicity and a consensus ranking of contamination. The order of consensus rank was determined by the average rank of each sludge-sediment mixture for all 15 parameters. For example, if a sludge-sediment mixture had the 3rd highest concentration for 5 parameters and the 4th highest for the other 10, its average rank would be $(3 \times 5) + (4 \times 10)/15 = 3.67$. The consensus rank was not calculated for other enrichment levels because data on metals were not collected for some samples. Finally, the data were combined from all six enrichment levels and rank correlation coefficients were calculated between mean survival and contaminant concentrations.

Results

Toxicity

There was a good dose-response relation in the sludge particulate bioassays allowing a rather precise determination of the LC50 (Table 2). Survival was not less than 85% (17 of 20 seeded amphipods) in any of the 15 control replicates for the three experiments. Average survival in the Yaquina Bay control sediment was 91%. In contrast, survival in sludge-sediment mixtures decreased well below control values in proportion to the increase in percent total volatile solids (TVS) due to sludge addition. The LC50 data permit a comparison of the relative toxicity of the six samples. The least toxic sludge came from the Waldport treatment plant. The Waldport LC50 was a TVS increase of 2.83% above background. Sludges from the Los Angeles County and Los Angeles City treatment plants had relatively high toxicity. No partial mortalities greater than 50% were obtained in the bioassays on Los Angeles samples. These data, therefore, do not provide exact LC50 estimates, but are adequate to establish relative toxicities of the different sludges. In both cases, the LC50 was less than 0.1%

Table 2. Mean survival of *Rhepoxynius abronius* in sediment organically enriched with sewage sludge. Twenty amphipods were seeded in each replicate

Percent increase in total volatile solids from sludge addition	Mean number of survivors after ten days exposure Sludge source					
	Waldport, OR	Newport, OR	Los Angeles City, CA	Los Angeles County, CA	Staten Island, NY	Middlesex County, NJ
0.0 (Control)	17.8[a]		18.2[a]		18.6[a]	
0.0625					19.0	19.0
0.125	18.2	17.0	4.8	0.4	19.0	17.2
0.25	17.2	12.6	1.4	0	17.6	16.6
0.5	15.2	3.4	0	0	9.6	11.2
1.0	14.6	0.2	0	0	0.6	0
2.0	10	0	0	0	0	0
4.0	8	0	0	0	0	0
LC_{50} (Percent total volatile solids)	2.83	0.28	0.08	0.07	0.44	0.42
95% Confidence limits	1.75–6.83	0.25–0.31	0.06–0.10	0.03–0.09	0.37–0.52	0.33–0.52

[a] Each of the three experiments included one control and two sludge treatments

TVS above background. The other three sludge samples had intermediate toxicity. LC50s were 0.28% TVS for Newport, 0.42% TVS for Middlesex County, and 0.44% TVS for Staten Island sources.

Reburial Success

Failure to rebury was rare. Only 17 of 1,465 survivors (1.2%) were unable to rebury. There was no significant difference in the proportion of reburial failures among survivors in control sediment (2/273 failed) and in sludge-sediment mixtures (15/1192 failed) ($\chi^2 = 0.18$, $p > 0.5$). However, all 15 failures in the sewage enriched sediment were among 366 amphipods from bioassay chambers in which survival was relatively low (<75%). There were no reburial failures among 826 amphipods from enriched sediment chambers in which survival was greater than 75%.

Chemistry

Dissolved oxygen (DO) and pH of the seawater in the beakers varied 7.0–9.7 mg/L and 7.8–9.0, respectively. Aeration minimized DO differences between treatments due to the oxygen demand of the sludge-sediment mixtures. Results of chemical analyses of sediment from the 2% TVS treatment at the initiation of the experiments illustrate differences between control and enriched sediment and the effects of the different sludge sources on chemical contamination (Table 3). The mean observed TVS increase (1.96%) was very close to the nominal increase (2.0%) for this treatment. Since TVS was experimentally controlled, it is not surprising that it had the lowest coefficient of variation of all sediment parameters measured. The sludge addition caused other parameters related to organic enrichment (Eh, percent solids, total organic carbon) to change as expected with relatively small differences between sludge sources (Table 3). The mean total organic carbon content increased by a factor of 5.4 over control conditions. Percent solids decreased slightly. The redox potential decreased from 329 to 382 mv in control sediment to 84 to 159 mv in the enriched sediment.

The ammonia nitrogen content of interstitial waters increased markedly in all sludge-sediment mixtures (Table 3). In contrast, the concentration of free sulfides in interstitial waters was not affected in a consistent pattern by the sludge addition.

The 2% TVS enrichment increased hydrocarbon and total oil/grease concentrations by a factor of 6 to 30 above control levels. The oil/grease concentration was greatest in sediment mixed with sludge from Los Angeles County, Newport, and Los Angeles City.

The degree of metal increase in the sludge-sediment mixtures depended on the metal species and sludge source (Table 3). Increases in Cr and Ag were relatively small (<0.3 μg/g) with little difference between sources. Average increases in Cd, Pb, and Cu were moderate (0.4–0.65 μg/g). The Middlesex County mixture was relatively high for each of these three metals. Cd was also high in the Los Angeles City mixture. The greatest increases in metal concentrations were for Ni and especially Zn. Ni was above 13 μg/g for both the Los Angeles County and Los Angeles City mixtures. Zinc was

Table 3. Chemical conditions at initiation of experiments in sludge-sediment mixtures with a 2% total volatile solids increase due to sludge addition

Source (Experiment number)	Eh (mv)	Solids (%)	Total Volatile Solids (%)	Total organic carbon (g/kg)[a]	Interstitial Water		Hydrocarbon oil/grease	Total oil/ grease	Cd	Pb	Zn	Cr	Cu	Ni	Ag
					Ammonia nitrogen (mg/L)	Free sulfides (mg/L)					(μg/g)[a]				
Waldport (1)	101	63.0	3.72	8.5	11.9	2.10	421	1054	0.11	0.60	7.1	0.58	0.40	1.3	0.53
Newport (1)	130	67.8	3.91	8.8	57.6	4.30	1824	2375	0.12	0.71	11.6	0.30	0.91	2.4	0.77
Los Angeles County (2)	84	66.1	3.07	13.4	12.4	0.77	2350	2597	0.59	1.00	25.9	0.47	0.77	13.3	0.34
Los Angeles City (2)	97	67.0	3.23	9.6	31.0	2.67	1608	2097	2.00	0.93	25.0	0.22	0.86	13.6	0.29
Staten Island (3)	159	67.3	3.45	8.7	24.6	0.67	963	1564	0.19	0.82	20.4	0.33	1.60	4.7	0.26
Middlesex County (3)	138	68.1	3.17	7.2	22.0	1.08	547	1234	1.50	1.40	36.0	0.56	3.00	1.6	0.34
Yaquina Bay Control															
Experiment 1	382	71.7	1.76	1.6	1.3	1.76	70	114	0.13	0.59	0.18	0.24	0.78	0.3	0.55
Experiment 2	340	72.8	1.28	1.6	0.3	3.01	131	168	0.07	0.47	0.48	0.14	1.10	1.6	0.17
Experiment 3	329	74.6	1.37	1.2	0.05	1.43	32	64	0.10	0.46	0.10	0.43	0.30	1.0	0.40
Difference between sediment-sludge mixture and control sediment															
Mean[b]	(232)	(6.5)	1.96	7.9	26.0	(0.14)	1207	1705	0.65	0.40	20.75	0.14	0.53	5.0	0.05
Standard deviation	42.5	1.6	0.14	2.0	16.7	1.57	733	600	0.87	0.33	10.4	0.17	1.23	5.4	0.14
Coefficient of variation	18.3	24.6	7.4	25.6	64.0	1161.4	60.7	35.2	133.8	81.8	50.3	121.1	233.0	107.2	294.7

[a] Dry weight

[b] Values in parentheses represent a reduction from control condition

Table 4. Significant rank correlations (Kendall's coefficient) between toxicity (based on LC_{50}) to *Rhepoxynius abronius* and chemical parameters in sludge-sediment mixtures

	Chemical Parameter							
Percent total volatile solids from sludge addition	Solids %	Total volatile solids %	Hydrocarbon oil/grease	Total oil/ grease	Cadmium	Zinc	Nickel	Consensus rank for 15 parameters
0.125	.65[b]		.59[b]	.48[a]				—
0.25	.51[b]		.51[a]	.43[a]				—
0.5	.42[b]		.70[b]	.67[a]	.53[a]	.55[a]		—
1.0	.66[b]		.51[a]	.40[a]				—
2.0			.48[a]	.54[b]	.50[a]	.44[a]	.60[a]	.66[b]
4.0		−.41[a]	.60[b]	.60[a]				—

[a] $p < 0.05$, [b] $p < 0.01$

There were no significant correlations at any enrichment level for Eh, total organic carbon, ammonia nitrogen, free sulfides, lead, chromium, copper, and silver

The consensus rank was calculated only for the 2% TVS chemical data set

above 20 μg/g for Los Angeles County, Los Angeles City, Staten Island, and Middlesex County mixtures. Metal enrichment was generally lowest for Waldport and highest for Middlesex County sludges.

Chemical characteristics of sludge-sediment mixtures for a single sludge source were generally proportional to the level of organic enrichment. Exceptions were free sulfides and metals for those sludge sources with low metal concentrations (*e.g.*, Waldport).

Correlation Between Toxicity and Chemical Contamination

Only 22 of the 90 rank correlations calculated between toxicity and chemical contamination were statistically significant ($p < 0.05$) (Table 4). Hydrocarbon oil and grease and total oil and grease were the only two parameters whose correlation with toxicity was statistically significant at all six enrichment levels. The correlation was significant for no more than 5 of the 15 parameters at any single enrichment level. However, the coefficient for the concensus or average ranking of all 15 parameters was highly significant at the 2% TVS enrichment level.

Correlation coefficients between mean survival and chemical parameters were also calculated for the combined data from all 6 enrichment levels (Table 5). The coefficients were significantly negative for all parameters except copper, chromium, free sulfides, and silver. The scatter diagrams for cadmium-survival (Figure 1) and TVS-survival relations (Figure 2) are representative of the significant results. Most of the points on the scatter diagram are arranged in an "L"-shaped pattern. The vertical segment of the "L" represents the broad range in survival that occurred at low (near control) concentrations of cadmium and total volatile solids. The horizontal segment of the "L" represents low survival over a broad range of cadmium and total volatile solids concentrations.

Table 5. Significant rank correlations (Kendall's coefficient) between mean survival of *Rhepoxynius abronius* and chemical parameters for combined data from all total volatile solids enrichment levels

Chemical parameter	Correlation coefficient
Hydrocarbon oil/grease	−.72[a]
Total oil/grease	−.69[a]
Zinc	−.60[a]
Cadmium	−.55[a]
Eh	−.55[a]
Nickel	−.53[a]
Ammonia nitrogen	−.50[a]
Total organic carbon	−.49[a]
Total volatile solids	−.47[a]
Lead	−.40[a]
Percent solids	−.27[a]
Chromium	−.10 ns
Copper	−.05 ns
Free sulfides	.00 ns
Silver	.13 ns

[a] $p < 0.01$, ns not significant $p > 0.05$

Ranks for Eh and percent solids were reversed, because these parameters are inversely proportional to organic enrichment and contamination

Discussion

Mixtures of sewage effluents and freshwater or seawater are not very toxic to pelagic organisms. The

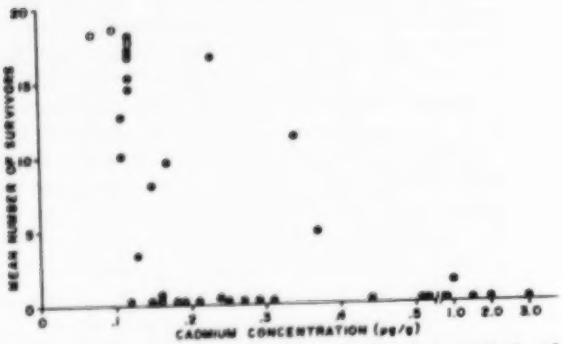

Fig. 1. Relation between *Rhepoxynius abronius* survival and cadmium concentration for sludge-sediment mixtures. Negative correlation for data from all enrichment levels and all sludge sources is highly significant ($\tau = -0.55$, $p < 0.01$). ○ control sediment, ● control sediment plus sludge

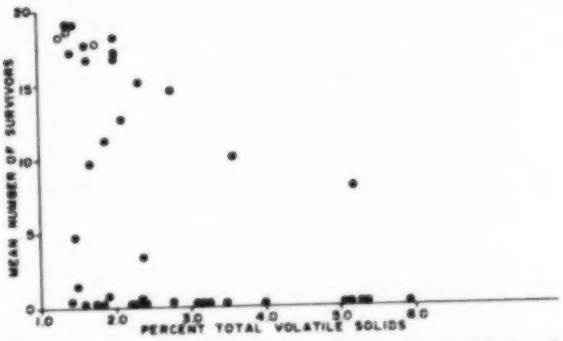

Fig. 2. Relation between *Rhepoxynius abronius* survival and percent total volatile solids for sludge-sediment mixtures. Negative correlation for data from all enrichment levels and all sludge sources is highly significant ($\tau = -0.47$, $p < 0.01$). ○ control sediment, ● control sediment plus sludge

96-hr LC50 of effluents from primary and secondary treatment is usually greater than 15% and sometimes exceeds 100% for freshwater and euryhaline estuarine fishes (Esvelt *et al.* 1973; Stober *et al.* 1978). More sensitive tests based on reductions in fertilization success, echinochrome synthesis, and development of sea urchin eggs have shown effects at 1 to 7% effluent concentrations (Oshida *et al.* 1981, 1982; Dinnel *et al.* 1981). Sewage sludges are more toxic than primary and secondary effluents, but Oshida *et al.* (1982) found that water collected within 100 m of a large sludge outfall had no effect on sea urchin development. The low toxicity of sewage wastes, rapid dilution achieved by outfall diffusers, and dispersion by currents all act to minimize the potential for acute toxic effects in the water column. Of greater concern is the potential toxicity that may result from the accumulation of toxic materials in benthic ecosystems.

Benthic effects can be caused by either organic enrichment of sediment or the toxic action of chemicals associated with sewage particulates. Organic enrichment effects are well known. Conceptual models exist for changes in the diversity and abundance of the macrobenthos as a function of sediment organic loading (Pearson and Rosenberg 1978). Equations exist for predicting the Infaunal Trophic Index (a measure of macrobenthic species composition and density) in relation to the mass emission rate of suspended solids from sewage outfalls in the Southern California Bight (Mearns and Word 1982).

Relatively little is known about potential toxic effects of chemical contamination near sewage outfalls, although faunal surveys around outfalls have provided circumstantial evidence for toxicity. Pearce (1972), Botton (1979), and Steimle *et al.* (1982) believed that sediment toxicity could explain the reduced abundance of crustaceans, particularly juvenile amphipods and brachyurans, at the sludge dumping site in the New York Bight. Bascom *et al.* (1979) discriminated infaunal responses to toxicants and organic enrichment on the Palos Verdes Shelf near the Los Angeles County outfalls. Major changes in biomass, density, and species composition reflected succession toward deposit feeding assemblages in response to organic enrichment. Bascom *et al.* (1979) attributed two impacts to toxic effects: the absence of echinoderms and small crustaceans, and reduced growth rate of the clam *Parvilucina tenuisculpta*. Swartz *et al.* (1981) reported that sediment toxicity was spatially correlated with contaminant concentrations on the Palos Verdes Shelf and that phoxocephalid amphipods were absent where sediment was significantly more toxic to *Rhepoxynius abronius* than uncontaminated controls.

The present results provide the first experimental laboratory demonstration of the toxicity of sewage sludge incorporated into sediment. The great differences between sludge sources in *Rhepoxynius abronius* mortality at comparable levels of total volatile solids (TVS) addition clearly indicates that toxicity is related to chemical contamination rather than organic enrichment (Table 2). If enrichment were the dominant factor, the TVS LC50 estimates should be similar for all sludges. Instead, the LC50 estimates are forty times greater for Waldport than for Los Angeles County sludge.

The distinction between chemical and organic enrichment effects is important from a regulatory perspective. Enrichment has the potential to increase the productivity of the sea as long as the loading remains within the assimilative capacity of the system. Although there are uncertainties about the ability of altered benthic assemblages in areas

of enrichment to support desirable fishery species (Boesch 1982), increases in species richness, density, and biomass are well documented near sewage disposal sites (Anger 1975; Pearson and Rosenberg 1978; Dauer and Conner 1980; Bascom 1982; Mearns and Word 1982). If the assimilative capacity of the system is exceeded, coastal benthic conditions will respond rapidly to a reduction in organic loading (Smith 1974). In contrast, the accumulation of toxic chemicals in sediment will not benefit benthic ecosystems. These pollutants are often persistent and remain in sediment long after sources have been controlled.

This sediment bioassay proved successful in determining the relative toxicity of sewage sludge from different wastewater treatment facilities. The LC50 for total volatile solids increase due to sludge addition was highest for sludge from principally domestic sources in Waldport, Oregon and lowest for sludge with greater industrial input in Los Angeles County and City. In addition to comparing the toxicity of sludges from different sources, this method could be used to compare sewage particulates generated from different treatment processes or collected at different times from the same treatment facility.

The results should not be extrapolated uncritically to field situations. There is an inherent limitation in the ability of any single species, short-term laboratory bioassay to accurately simulate natural conditions (Carriker *et al.* 1982; Livingston 1979). Bioassays are usually designed to detect potential problems, not to measure impacts (Lee and Jones 1982). In particular, the method of mixing sludge with sediment does not simulate the fate of sewage particulates in the ocean. That fate is determined by hydrodynamic dispersion, settling characteristics, benthic deposition and resuspension, bioturbation, sedimentation, and biological and chemical degradation (Cook 1979; Jackson 1982; Koh 1982; Bascom 1982). Only about 10% of the solids settle within a 2 km^2 area near the sludge discharge in Santa Monica Bay, California and only 30% settle within two weeks of discharge (Bascom *et al.* 1980). This dispersion produces a spatial segregation of particulates based largely on size and falling rate. Benthic bioassays of sludges collected at the treatment plant are therefore useful in determining relative toxicities but cannot reliably indicate the level of contamination that would be acutely toxic in the field.

One aspect of this bioassay that is very relevant to field conditions is the choice of the test animal, *Rhepoxynius abronius*. This species and other phoxocephalid amphipods are small infaunal organisms that are often abundant in clean sands and are quite sensitive to sediment contamination. Swartz *et al.* (1979) found that *R. abronius* was more sensitive to contaminated sediment than four other benthic invertebrates. Mearns and Word (1982) included three phoxocephalid genera among taxa representative of control or background areas near sewage outfalls in the Southern California Bight. Swartz *et al.* (1982) found phoxocephalids were ubiquitous in the deeper parts of Commencement Bay, Washington, but absent from adjacent highly industrialized waterways. Amphipods are the principal benthic prey of many fishes (Boesch 1982), and phoxocephalid amphipods, in particular, were the dominant burrowing invertebrate prey of seven of eight fish species studied by Manzanilla and Cross (1982). Phoxocephalids also have an important community function as predators of larval and juvenile benthic invertebrates (Oliver *et al.* 1982). Thus, phoxocephalid amphipods are ideal benthic bioassay species because of their ecological significance and sensitivity to sediment quality.

Both mortality and sublethal effects on reburial behavior were recorded in the sewage sludge bioassays. The ability of an infaunal species to quickly rebury has obvious adaptive significance. Very few of the animals that survived were unable to rebury in clean sand. However, most of the amphipods that were unable to rebury came from treatments in which mean mortality was relatively high. These animals were subjected to sublethal stresses that may have increased their vulnerability to predation under natural conditions.

All statistically significant rank correlations between mean survival and contaminant concentrations were negative when data from all organic enrichment levels were pooled (Table 5). This result provides little insight into causal relations. The negative coefficients demonstrate only that survival decreased as more sludge was added to clean sediment. Parameters that showed no significant correlation in this analysis (copper, chromium, free sulfides, silver) were not greatly affected by the sludge addition (Table 3). Most points on the scatter diagrams for the survival-contaminant correlations that were significant are arranged in an "L"-shaped pattern (Figures 1, 2). This pattern shows that the concentration of individual toxic compounds was sometimes very low in highly toxic sludge-sediment mixtures. Different compounds or different combinations of stresses must have contributed to observed effects in different treatments. Unfortunately, many of the stresses imposed by complex waste mixtures may be unknown or unanalyzed. Even if all contaminants were known, their interactions are poorly understood (Malins and Collier 1981; Negilski *et al.* 1981).

Rank correlations between toxicity and the concentration of specific chemicals in sediment samples at the same enrichment level were usually not significant (Table 4). This result also suggests that different, multiple, or unknown factors were responsible for toxicity in the different treatments. The highly significant correlation between toxicity and the consensus rank of contamination indicates that the more toxic sludges had a greater overall chemical contamination at comparable levels of organic enrichment.

Two parameters (total and hydrocarbon oil/grease) were exceptions to the general lack of statistical significance in these correlations with toxicity (Table 4). Oil/grease concentrations also showed the highest correlations with survival for data pooled from all treatments (Table 5). Effects of oil/grease on the texture or other physical properties of sediment are probably not responsible for toxicity. High toxicity was observed in some treatments with low oil/grease concentrations (*e.g.*, Los Angeles County .125% TVS, 258 μg/g total oil/grease, 2.0% survival) while less toxicity occurred in some mixtures that contained much oil and grease (*e.g.*, Waldport 4.0% TVS, 1564 μg/g total oil/grease, 40.0% survival). High survival of *Rhepoxynius abronius* has also been recorded in sediment with very high oil/grease concentrations collected near sewage outfalls (6020 μg/g total oil/grease, 92.0% survival; Swartz, unpublished data). Oil/grease analyses reflect the concentration of many organic compounds (APHA 1980) and may integrate the overall level of contamination. Research on crude and refined petroleum hydrocarbons also indicates that the toxicity of the oil/grease component of complex waste mixtures is more dependent on its quality than quantity (Anderson *et al.* 1974; Buikema *et al.* 1981).

The treatment plants from which sludges were obtained have different industrial, commercial, and residential inputs. Chemical characteristics of the sludge-sediment mixtures therefore varied greatly (Table 3). This variation is probably typical of most complex wastes disposed in the ocean. The present results are not unique: the scope of chemical analyses is always limited, interactions between many contaminants are impossible to predict, and correlations between specific chemicals and toxicity are therefore likely to be non-significant or spurious because of gradient or dilution phenomena. It is concluded that the sediment bioassay is a method of pollution assessment whose validity is not dependent on correlation with sediment chemistry.

The results are similar to those of Tsai *et al.* (1979) who found no significant correlation between the acute toxicity of Baltimore Harbor sediment to the fish, *Fundulus heteroclitus*, and the concentration of a variety of metals, PCBs, and oil/grease (hexane extracts). They reported oil/grease was "best in this respect" but not statistically significant. We concur with their conclusion that generally no single chemical component can be used to indicate sediment toxicity.

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